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Rapid, effective deprotection of *tert*-butoxycarbonyl (Boc) amino acids and peptides at high temperatures using a thermally stable ionic liquid†

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A method for high temperature Boc deprotection of amino acids and peptides in a phosphonium ionic liquid is described. The ionic liquid had low viscosity, high thermal stability and demonstrated a beneficial effect. The study extended the possibility for extraction of water soluble polar organic molecules using ionic liquids. Trace water significantly improved product purity and yield, while only 2 equiv. TFA led to deprotection within 10 min. The trityl group was also deprotected.

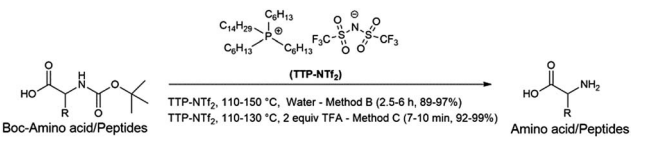
The development of simple, effective and environmentally compliant organic transformations are increasingly sought after as a means to drive sustainable processes.[1] Ionic liquids (ILs) have appeared in the literature as a new class of designer solvents and have been found to be attractive for organic synthesis.[2] They offer several advantages over traditional solvents including negligible vapor pressure (safety), recyclability (lowering cost) and modulation of properties (scope).[3]

Sequential protection and deprotection of amine functional groups play a significant role in organic synthesis.[4] The *tert*-butoxycarbonyl (Boc) group is one of the more widely used amine protecting group.[5] The most common method for its deprotection uses TFA and generally requires large excesses (TFA : CH_2Cl_2 (1 : 1)), reaction times ranging from 2–16 h depending on the substrate and a tedious purification process.[6] Another well-established method for faster (~10–30 min) and selective deprotection (in most cases) of Boc group utilizes (4 M) HCl/dioxane.[7] In a classic publication, Wang *et al.* reported deprotection of *N*-Boc aliphatic, aromatic and heterocyclic amine substrates using boiling water as a reaction medium, indicating the role of water as a dual acid/base catalyst at elevated temperature.[8] However, reaction times with water insoluble substrates could be significantly longer, when water is the only solvent. A few Boc deprotections have also been reported in subcritical water (150 °C < T < 370 °C, 0.4 < p < 22 MPa) to take advantage of the higher dissociation of water.[9] Extraction of water soluble organic molecules using ionic liquids have been explored with the advent of [BMIM]PF_6 ionic liquid.[10] Recently, Majumdar *et al.* reported deprotection of several *N*-Boc protected aromatic and heteroaromatic compounds using imidazolium based protic ionic liquid and also discussed the selectivity of their approach.[11] Interestingly, use of phosphonium based ionic liquids in organic synthesis has been very limited. This is surprising, given some of their interesting properties like lack of an acidic proton (which may lead to carbene formation), generally lower density than water (helpful in liquid–liquid extraction), tolerance towards air, moisture and high thermal stability.[12]

To the best of our knowledge, this is the first report on a generalized Boc-deprotection of natural amino acids and peptides in ionic liquid media. There are challenges in purification of water soluble compounds involving many ILs traditionally used in organic synthesis (due to their own aqueous solubility). This problem is circumvented by the current approach.

The current work, utilizes trihexyltetradecylphosphonium bis(trifluoromethane)sulfonimide (TTP–NTf_2) ionic liquid as a water insoluble solvent having a relatively low kinematic viscosity of 218.6 cSt @ 30 °C,[12] density of 1.065 g cm^{-3},[12] high thermal stability (~380 °C),[12] broad substrate solubility and thermal range (−22 to 350 °C).[12]

Three different methods were employed to liberate the built in gaseous entity in the Boc group (Scheme 1 and Table 1).



Scheme 1 Boc deprotection of amino acids using phosphonium ionic liquids.

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Table 1 Deprotection of *N*-Boc amino acid and peptide substrates

#	Substrate	Method A[a] neat + Δ, yield (%), time (h)	Method A product	Method B[b] H_2O + Δ, yield (%), time (h)	Method B product	Method C[c] TFA + Δ, yield (%), time (h)	Method C product
Amino acids[a,b,c,d]							
1	*N*-Boc-L-Gly-OH	71% (5 h)	H-L-Gly-OH	96% (3 h)	H-L-Gly-OH	93% (7 min)	H-L-Gly-OH
2	*N*-Boc-L-Ala-OH	67% (5 h)	H-L-Ala-OH	91% (3 h)	H-L-Ala-OH	98% (7 min)	H-L-Ala-OH
3	*N*-Boc-L-Val-OH	29% (6 h)	H-L-Val-OH	92% (6 h)	H-L-Val-OH	93% (10 min)	H-L-Val-OH
4	*N*-Boc-L-Leu-OH	D[d] (6 h)	NA	89% (6 h)	H-L-Leu-OH	98% (10 min)	H-L-Leu-OH
5	*N*-Boc-L-Ile-OH	D[d] (6 h)	NA	94% (6 h)	H-L-Ile-OH	98% (10 min)	H-L-Ile-OH
6	*N*-Boc-L-Phe-OH	20% (6 h)	H-L-Phe-OH	93%(5 h)	H-L-Phe-OH	95% (10 min)	H-L-Phe-OH
7	*N*-Boc-L-Pro-OH	D[d] (6 h)	NA	95% (5 h)	H-L-Pro-OH	96% (10 min)	H-L-Pro-OH
8	*N*-Boc-L-Met-OH	D[d] (6 h)	NA	92% (5 h)	H-L-Met-OH	97% (10 min)	H-L-Met-OH
9	*N*-Boc-L-Thr-OH	D[d] (6 h)	H-L-Thr-OH	90% (5 h)	H-L-Thr-OH	92% (10 min)	H-L-Thr-OH
10	*N*-Boc-L-His-OH	D[d] (6 h)	NA	92% (6 h)	H-L-His-OH	92% (10 min)	H-L-His-OH
11	Boc-L-Lys(Boc)-OH	D[d] (6 h)	NA	91% (6 h)	H-L-Lys-OH	93% (11 min)	H-v-Lys-OH
12	*N*-Boc-L-Asp-OH	87% (5 h)	H-L-Asp-OH	97% (2.5 h)	H-L-Asp-OH	99% (10 min)	H-L-Asp-OH
13	*N*-Boc-L-Glu-OH	27% (5 h)	H-L-Glu-OH	93% (4 h)	H-L-Glu-OH	96% (10 min)	H-L-Glu-OH
Peptides[e,f,g]							
14	*N*-Boc-Ala-Ala-OH	13%	—	92% (2.5 h)[f]	H-Ala-Ala-OH	94% 10 min[g]	H-Ala-Ala-OH
15	*N*-Boc-Gly-Val-OH	IC[e]	—	94% (2.5 h)[f]	H-Gly-Val-OH	95% 10 min[g]	H-Gly-Val-OH
16	Boc-Gly-His(Trt)-Lys(Boc)-OH	IC[e]	NA	92% (2.5 h)[f]	H-Gly-His-Lys-OH	95% 7–8 min[g]	H-Gly-His-Lys-OH

[a] TTP–NTf_2, 150 °C, 5–6 h (incomplete reaction with unreacted starting material for entry 1, 2, 3, 6, 12 and 13. For entry 14 A, trace condensation by product observed along with unreacted starting material). [b] TTP–NTf_2, 12–14% water as additive (based on TPP–NTf_2), 150 °C, 2.5–6 h. [c] TTP–NTf_2 2 equiv. TFA as additive (based on substrate), 130 °C, ~10 min. [d] D (decomposed), TTP–NTf_2 150 °C, 6 h. [e] IC (trace product with unreacted starting material and some decomposition observed), TTP–NTf_2, 120 °C, 3 h. [f] TTP–NTf_2, 110 °C, 12–14% water as additive (based on TPP-NTf_2), 2.5 h. [g] TTP–NTf_2, 2 equiv. TFA as additive based on substrate, 110 °C, 7–10 min. Yields reported are isolated.

Simple thermal treatment of Boc protected amino acids at around 150 °C gave the desired product for selected substrates, however the yields were not satisfactory with incomplete conversions and some product decomposition was observed (Table 1, 1A–16A).

Addition of small amount of water led to significant improvement in yield and product purity as evident in (Table 1, 1B–16B). Deprotection of Boc-L-aspartic and glutamic acid (having two free acid groups) conversion took the least time. It is well known that quaternary ammonium and phosphonium cations act as phase transfer agents.[13] Therefore, reactions were also performed in tetrabutylammonium NTf_2 (mp 84–85 °C) with *N*-Boc-L-Ala-OH as substrate under reaction conditions identical to method B, which gave alanine in 97% yield. A similar reaction with butylmethylimidazolium NTf_2 resulted in incomplete conversion with 67% of L-alanine as product. These observations may indicate the possibility of phosphonium cation acting as a phase transfer catalyst, while acidic pH in the aqueous phase would drive deprotection of Boc group at high temperature. Lewis acidic type interaction of carbonyl group with imidazolium based ionic liquids have been indicated in earlier reports.[11,14] For phosphonium ionic liquids, especially in the absence of water as a separate phase, similar interactions have been suggested.[15–17] This however does not appear to be operative in the present case.

In the third method variation, (Table 1, 1C–16C) addition of only 2 equiv. TFA resulted in accelerated deprotection within 10 min in almost all the cases studied compared to conventional methods that requires 2–5 h and uses high concentrations of TFA (generally 1 : 1 TFA : CH_2Cl_2).[6] The method was extended for the deprotection of Boc groups of dipeptides and two Boc and a trityl group for a tripeptide. However, some condensation products were observed for entry 14B which was avoided by performing the reaction at 110 °C for 2.5 h.

In summary, the current work provides a simple and convenient procedure for Boc-deprotection reaction in ionic liquid media. The lower viscosity, broad substrate solubility and thermal stability of the ionic liquid provides scope for carrying out high temperature organic reactions and opens up the possibility of extraction and purification for water soluble organic compounds using ionic liquids. The rapid deprotection; using only two equivalent of TFA (little excess of which is mostly removed during the water distillation under reduced pressure) allowed the whole process to be very efficient. The various amino acids were extracted without any chromatographic purification (as it can be challenging to separate ionic liquid and water miscible organic compounds in the normal phase mode using silica gel chromatography). Also the said ionic liquid from all batches were pooled together and recycled for further use by column purification using silica gel chromatography (ethylacetate/hexane 70 : 30 to ethyl acetate 100%). Reactions performed with the recycled ionic liquid, (92% recovery and characterized by NMR spectroscopy) gave similar efficiencies. Study of efficient and waste free high temperature organic transformations in phosphonium based ionic liquid

and their role in solvent assisted catalysis are underway in our laboratory. We would like to thank Dr Zachary S. Breitbach and Mohsen Talebi for technical support. We also acknowledge funding for this work from Robert A. Welch Foundation (Y0026).

Method A (neat reaction in TTP–NTf_2)

A mixture of (0.25 g, 0.32–1.43 mmol) *N*-Boc protected amino acid/peptides and TTP–NTf_2 (7 g, 9.16 mmol) was stirred (~150 rpm) for 5–6 h at 150 °C in a round bottom flask (50 mL) equipped with a reflux condenser. Completion of reaction was monitored by TLC using ethyl acetate/methanol (95 : 5) and ninhydrin as staining agent. On cooling, the reaction mixture was transferred into separating funnel with 3 × 10 mL (CH_2Cl_2/water 1 : 1). In addition, CH_2Cl_2 (100 mL) and water (50–80 mL, depending on the substrate) was added to the separating funnel. The layers were allowed to separate after shaking and the organic layer (containing the ionic liquid) was removed. To the aqueous layer (containing the amino acids) fresh CH_2Cl_2 (2 × 75 mL) was added to ensure removal of any trace of ionic liquid remaining. The aqueous layer was evaporated to dryness to obtain the product. In most of the cases, this gave us the purified amino acid, but otherwise a final washing with ~5 mL isopropanol (using centrifuge). All the products were characterized by ^{1}H NMR, ^{13}C NMR. ESI-MS was done in some cases.

Method B (TTP–NTf_2 and water)

To a mixture of (0.25 g, 0.32–1.43 mmol) *N*-Boc protected amino acid/peptides and TTP–NTf_2 (7 g, 9.16 mmol) was added deionized water 12–14% (based on TTP–NTf_2). The resulting mixture was stirred (~200 rpm) for 2.5–6 h at 150 °C (for amino acids) and 2.5 h at 110 °C (for peptides). The remaining work-up is similar to method A. All the products were characterized by ^{1}H NMR, ^{13}C NMR. ESI-MS was done in some cases.

L-Histidine and L-lysine tends to form emulsions especially for method B. They were centrifuged for 5 min at around 5000 rpm (the Drucker Co., Model 614 B) to obtain the purified compound. For peptides, the water was generally removed at ~40–45 °C under reduced pressure.

Method C (TTP–NTf_2 and TFA)

To a stirring mixture of (0.25 g, 0.32–1.43 mmol) *N*-Boc protected amino acid/peptides and TTP–NTf_2 (7 g, 9.16 mmol) was added TFA (2 equiv., based on substrate). The resulting mixture was stirred (~150 rpm) for 7–10 min at 100 °C (for peptides) and 130 °C (for amino acids). The remaining procedure is similar to method A.

For peptides, the water was removed by either freeze drying, air drying at higher flow in a crystallizing dish using an inverted funnel at room temperature or direct precipitation of amino acid by adding acetone. All the products were characterized by ^{1}H NMR, ^{13}C NMR. ESI-MS was done in some cases.

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